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Review

Cell-based approaches to joint surface repair: a research perspective

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SUMMARY

Repair of lesions of the articular cartilage lining the joints remains a major clinical challenge. Surgical interventions include osteochondral autograft transfer and microfracture. They can provide some relief of symptoms to patients, but generally fail to durably repair the cartilage. Autologous chondrocyte implantation has thus far shown the most promise for the durable repair of cartilage, with long-term follow-up studies indicating improved structural and functional outcomes. However, disadvantages of this technique include the need for additional surgery, availability of sufficient chondrocytes for implantation, and maintenance of their phenotype during culture-expansion. Mesenchymal stem cells offer an attractive alternative cell-source for cartilage repair, due to their ease of isolation and amenability to *ex vivo* expansion while retaining stem cell properties. Preclinical and clinical studies have demonstrated the potential of mesenchymal stem cells to promote articular cartilage repair, but have also highlighted several key challenges. Most notably, the quality and durability of the repair tissue, its resistance to endochondral ossification, and its effective integration with the surrounding host tissue. In addition, challenges exist related to the heterogeneity of mesenchymal stem cell preparations and their quality-control, as well as optimising the delivery method. Finally, as our knowledge of the cellular and molecular mechanisms underlying articular cartilage repair increases, promising studies are emerging employing bioactive scaffolds or therapeutics that elicit an effective tissue repair response through activation and mobilisation of endogenous stem and progenitor cells.

Introduction

Traumatic lesions of the joint surface remain a major clinical challenge, due mainly to the poor self-healing ability of articular cartilage. If untreated, these joint surface lesions can lead to secondary osteoarthritis (OA). It is estimated that post-traumatic OA represents 13% of knee OA and 73% of ankle OA[1]. In a prospective study, it was reported that traumatic joint lesions in young adults double the risk of developing knee OA[2]. Hence, symptomatic chronic full-thickness defects of the knee joint surface require intervention for symptom relief and to prevent possible evolution towards OA. Tissue engineering and regenerative medicine offer the potential for a long-term solution *via* biological repair or replacement of the damaged joint tissues[3]. This review discusses the current status of cell-based approaches to repair traumatic joint surface lesions.

Current therapeutic interventions

Osteochondral autograft transfer system (OATS) and mosaicplasty involve the use of osteochondral plugs from non-weight-bearing regions of the joint to repair injured articular cartilage[4]. A study investigating the outcomes of over 800 mosaicplasties performed over a 10-year period showed promising results in tibial, patello-femoral and femoral condyle lesions but also discovered multiple cases of adverse events including the need for second-look arthoscopies, painful haemarthrosis, and mild-to-severe degenerative change within the joint[5]. An alternative surgical procedure is microfracture, which aims at creating a surgical communication between the joint space and the subchondral bone marrow. The theoretical basis for the cartilage repair is the release from the marrow space of mesenchymal stem cells (MSCs) and progenitors, which would then form a repair tissue. Significant pain relief and improved function of the glenohumeral joint, and improved function of the knee joint, were reported in case series of 16 and 72 participants, respectively[6,7]. While these procedures appear to alleviate pain and improve mobility, neither of these surgical techniques have been subjected to randomised, controlled trials, and as such their true effectiveness for achieving durable cartilage

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repair is unclear[4]. Although symptom relief appears to be independent of the quality of the repair tissue, a fibrocartilaginous repair tissue may not sustain a long-lasting therapeutic effect because of the high risk of secondary breakdown due to its poor mechanical properties. Marrow stimulation techniques such as microfracture promote a "callus-like" repair tissue, which can undergo degeneration over time with the formation of scar-like fibrous tissue or be replaced with bone[8,9]. Indeed, patients failing microfracture treatment do not seem to show a hyaline-like cartilage repair tissue[10].

Autologous chondrocyte implantation (ACI)

ACI involves obtaining a cartilage biopsy from a healthy area of the patient's articular cartilage, isolating and culture-expanding the chondrocytes, and implanting the culture-expanded chondrocytes within the cartilage defect, traditionally under a periosteal flap. ACI was first described in 1994 by Brittberg and colleagues, who reported symptomatic relief in 14 out of 16 patients with lesions of the femoral condyle at 2 years follow-up[11]. Since ACI was first approved by the US Food and Drug Administration in 1997, the technique has evolved into second and third generation ACI with the use of synthetic membranes and three-dimensional matrices[12]. The recently coined third generation technique, or matrix-induced ACI (MACI), involves the culture of the harvested autologous chondrocytes onto a three-dimensional biocompatible scaffold, which is subsequently implanted *via* either arthroscopy or open surgery[13].

Clinical trials have confirmed the good clinical outcome of ACI, but whether ACI is superior to other standard treatments in controlled prospective clinical trials is somewhat controversial. Superiority in terms of structural outcome was shown with ACI over mosaicplasty[14], while there was no clear difference in short-term (2 years) structural or clinical outcome with microfracture[15]. More recently, Saris *et al.* assessed 118 patients at 12 and 18 months following either ACI (using a characterised cell therapy product) or microfracture. Clinical outcome was similar in both groups but ACI was associated with increased structural repair[16]. At 5 years clinical outcomes were again comparable. However, ACI was statistically more effective in a subgroup of patients who had undergone the procedures close to presentation of symptoms[17]. Results from long-term follow-up studies are now beginning to be reported. At 10-year follow-up, in a population of 100 patients with large joint lesions, the ACI procedure showed a lower failure rate (17% vs 55%) and better functional outcome, compared to mosaicplasty[18]. Up to 20 years' follow-up have demonstrated that ACI is an effective and durable solution for the treatment of large joint surface lesions of the knee joint[19]. A key question that now remains is whether ACI intervention is effective in preventing secondary OA.

Nonetheless, ACI poses several challenges. Chondrocytes are harvested from a cartilage biopsy of a low load-bearing area of the same joint, thus requiring an additional surgical procedure. The amount of healthy cartilage available for chondrocyte harvesting can be limiting, and chondrocyte yields and *in vitro* proliferative capacity decrease with age[20], resulting in a limited number of cells available for transplantation, especially in older patients. Furthermore, chondrocytes have a tendency to dedifferentiate during culture-expansion to a fibroblast-like phenotype[21,22] and lose their capacity to form stable hyaline cartilage *in vivo*[23]. After ACI, remodelling and maturation of the cartilage repair tissue appear to occur over time[24]. It is tempting to speculate that, under specific conditions, in patients after ACI a regeneration process may occur after an initial phase of repair, but this is likely to require an optimal combination of high-quality chondrogenic cells and receptivity of the joint environment. It is therefore not surprising that the phenotype of culture-expanded chondrocytes, as well as the time between symptom onset and treatment, are important factors to influence clinical and structural outcomes[25]. The use of chondrocytes expanded under conditions that preserve their cartilage-forming potency and the phenotypic characteristics of articular cartilage (resistant to vascularisation and replacement by bone) may enhance potential for hyaline-like cartilage repair tissue formation leading to remodelling and, ultimately, joint surface regeneration[25]. The remodelling of the cartilage repair tissue is likely to be complex and delicate, hence susceptible to failure leading to the formation of a mechanically ineffective scar-like repair tissue, a doorway to secondary OA. Indeed, variability in structural outcome after ACI has been reported, with some patients showing repair tissue consisting of poorly differentiated and disorganised fibrocartilage[26].

MSC-based cartilage repair

MSCs are considered an attractive alternative cell-source for cartilage repair due to their relative ease of isolation, their amenability to *ex vivo* expansion while retaining stem cell properties, and their ability to give rise to chondrocytes. MSCs were first isolated and characterised from bone marrow[27–29]. Since then, MSCs have been isolated from most connective tissues[30] including, amongst others, periosteum[31–33], synovium[34], and adipose tissue[35].

Preclinical studies have demonstrated the potential for culture-expanded MSCs to promote repair of cartilage and subchondral bone and prevent secondary OA. In a study published almost 2 decades ago now, Wakitani *et al.* implanted either bone marrow or periosteal MSCs suspended in a collagen gel into full-thickness cartilage defects in the medial femoral condyle of rabbits. Hyaline-like repair tissue quickly formed which was superior to the fibrous tissue formed in defects treated with MSC-free collagen implants, although a progressive thinning and deterioration of the articular cartilage was observed over time, with biomechanical properties inferior to the native cartilage, and roughening of the articular surface[36]. Murphy *et al.* showed in goats in which the medial meniscus was excised and the anterior cruciate ligament was resected, that intra-articular injection of MSCs suspended in a sodium hyaluronan solution stimulated regeneration of the medial meniscus and decreased development of secondary OA as seen in untreated animals[37].

Results of clinical case reports and small clinical case series suggest that MSC therapy may improve cartilage repair[38–40], but to date, only one small controlled clinical trial of MSC therapy for cartilage repair has been reported. Wakitani *et al.*[41] implanted autologous bone marrow MSCs suspended in a collagen gel into 12 patients with knee OA undergoing high tibial osteotomy and covered the implanted cells with autologous periosteum. Another 12 patients underwent the same procedure but without MSCs. The MSC-treated group demonstrated improved arthroscopic and histological grading scores compared to the cell-free control group, as assessed up to an average of 42 weeks after the procedure. However, the repair tissue still appeared inferior to the surrounding native cartilage, with only 20–40% of cells showing a round to polygonal shape similar to chondrocytes of hyaline cartilage, and toluidine blue metachromasia mostly restricted to the middle/deep zone with lack of staining in superficial zone. Furthermore, there was no significant clinical improvement[41]. Similar histological results were reported in a case study by Kuroda *et al*[38]. Until results from large, prospective, randomised, controlled clinical trials become available, definitive conclusions regarding the clinical and structural benefits of MSC therapy for cartilage repair/regeneration cannot be drawn. Clinical trials comparing ACI with autologous

MSC implantation for the repair of joint surface lesions should shed light on the suitability of MSCs to replace chondrocytes as cell source for cartilage repair. Nejadnik *et al.*[42] reported on clinical outcomes of 72 patients who received either ACI or autologous bone marrow-derived MSC implantation. Clinical scores were similar between both groups up to 24 months after the procedure. Histological assessment of the repair tissue 1 year after the procedure was shown for one patient, showing the presence of a hyaline-like repair tissue, although full tissue morphology was unclear and no comparison with repair tissue from an ACI-treated patient was shown. Nevertheless, these data suggest that MSCs could be a suitable alternative cell source to chondrocytes at least on the basis on non-inferiority in terms of clinical outcomes[42], although longer-term follow-up and, ideally, more robust assessment of structural outcome are needed to draw definitive conclusions.

While preclinical and clinical studies have demonstrated the potential of MSCs to promote articular cartilage repair and, at least in some preclinical studies, decrease the development of secondary OA, these studies have also highlighted several key challenges; most notably, the quality and durability of the repair tissue, its resistance to endochondral ossification and replacement by bone over time, and its effective integration with the surrounding host tissue. In addition, challenges exist related to the heterogeneity of MSC preparations and their quality-control, as well as optimising the delivery method.

Regulating MSC differentiation and cartilage phenotype

Articular chondrocytes are now well-recognised as being able to form stable cartilage *in vivo* that is resistant to vascular invasion, calcification and endochondral ossification[23]. These properties are at least desirable, if not required, in a cellular product aiming at articular cartilage repair/regeneration. The molecular basis of chondrocyte stability, however, remains unknown.

Chondrogenic differentiation of MSCs *in vitro* using classical micromass/pellet high cell density assays attempts to reproduce the developmental chondrogenic limb anlage cascade of events. However, it typically induces an “unnatural” differentiation pathway resulting in concomitant expression of markers of hyaline as well as fibrous and hypertrophic cartilage[43,44]. Fibrous cartilage, characterised by high levels of collagen type I, is inferior to hyaline cartilage in mechanical properties, and is susceptible to degeneration and failure over time. Hypertrophic cartilage, characterised by expression of collagen type X, matrix metalloproteinase-13 (MMP-13), vascular endothelial growth factor (VEGF), alkaline phosphatase, and other hypertrophic chondrocyte markers normally found in growth plate cartilage but not in stable articular cartilage, undergoes mineralisation and remodelling by osteoclasts and osteoblasts in a process reminiscent of developmental endochondral ossification, leading to replacement of the cartilage by bone[45].

Studies comparing donor-matched bovine MSCs and articular chondrocytes for their cartilage-forming capacity *in vitro* using an agarose hydrogel system and stimulation with transforming growth factor beta (TGF-β) revealed that the amount and mechanical properties of the extracellular matrix produced by MSCs were inferior to those produced by chondrocytes, and plateaued with time, suggesting the diminished capacity is not the result of delayed differentiation[46–48]. In addition, in contrast to chondrocyte pellets, when *in vitro* MSC-derived cartilaginous tissues were transplanted ectopically in mice, depending on culture conditions and MSC type, they were either broken down and resorbed[45,49,50], or calcified, invaded by vasculature and ultimately remodelled to bone through a process mimicking endochondral ossification[44,45,50,51]. Although *in vitro* culture and ectopic implantation may not necessarily reflect the outcome following orthotopic transplantation susceptible to environmental cues, these studies highlight important intrinsic differences between articular chondrocytes and MSCs in the stability of cartilage that is produced. Uplift of the bone front at the expense of the overlying articular cartilage has been observed in osteochondral repair by bone marrow cells[52], and this has its clinical counterpart in intra-lesional osteophyte formation in patients after microfracture[53]. This phenomenon appears to be less frequent in patients treated with ACI[54], suggesting that an imprinted memory of articular chondrocytes could be sufficient to limit the advancement of the bone front, thus preserving the normal thickness of the repaired cartilage tissue. While remodelling of the MSC-derived cartilaginous tissue to bone may be ideal for bone repair through recapitulation of developmental processes[45,51], this is clearly undesirable for the durable repair of articular cartilage. There is thus a need for improved strategies to enhance cartilage properties and reduce fibrous and hypertrophic tissue formation.

Many pathways are involved in the regulation of chondrocyte hypertrophy, including parathyroid hormone-related protein (PTHrP)/Indian hedgehog (IHH), wingless/int (WNT)/β-catenin, and TGF-β/sma and mad-related family (SMAD) pathways, converging on runt-related transcription factor 2 (RUNX2) and myocyte enhancer factor 2C (MEF2C) to drive expression of hypertrophic genes. Modulation of these pathways to suppress hypertrophy of MSC-derived cartilage-like tissues has been explored, either directly for example by treatment with PTHrP[55–57], or indirectly through factors including hypoxia, co-culture with articular chondrocytes, epigenetic modulation, and biomaterial composition[58]. Such studies have demonstrated that modulation of chondrogenic hypertrophy is feasible, at least *in vitro*. Whether sustained suppression of hypertrophy and maintenance of a stable cartilage phenotype can be achieved *in vivo* using any of these approaches remains to be determined.

The solution to the challenge of stable-cartilage formation may come from the use of chondroprogenitors isolated from the articular cartilage itself[59,60]. By using a differential adhesion assay to fibronectin, the Archer lab has recently identified a population of chondroprogenitors from human articular cartilage with the ability to maintain chondrogenic potency upon extensive expansion, unlike full-depth chondrocytes that lost this ability after only seven population doublings. A proof-of-principle pilot study in a goat model *in vivo* demonstrated the apparent non-inferiority of these chondroprogenitors to form a cartilage-like repair tissue in a chondral defect when compared with full-depth chondrocytes, although both cell types resulted in repair tissue with a rough and irregular surface appearance suggesting that also in this study, true cartilage regeneration may not have been achieved[61]. Human studies are now awaited.

Heterogeneity of MSCs between tissues

Bone marrow has been the most commonly used tissue for extraction of MSCs and is currently regarded as the gold-standard MSC source for musculoskeletal tissue engineering approaches. It is not clear, however, whether bone marrow is the ideal source of MSCs for the repair of articular cartilage. Several studies have directly compared donor-matched MSCs from different tissues. Overall, synovium-derived MSCs appear to have the greatest chondrogenic ability *in vitro* when compared with MSCs from bone marrow, periosteum, adipose tissue, infrapatellar fat pad, and/or muscle, in humans[50,62–64], rats[65] and rabbits[66]. Following transplantation into full-thickness cartilage defects in rabbits, bone marrow, periosteal and synovial MSCs induced osteochondral repair, while adipose and muscle-derived MSCs failed to repair or

induced mostly fibrous tissue formation[66]. Most of these studies only compared the quantity of cartilaginous matrix produced without comparing the formed tissue qualitatively or assessing its stability, aspects which may have greater clinical relevance. In a recent study by Vinardell *et al.*, synovial and infrapatellar fat pad-derived MSC chondrogenic pellets appeared to undergo fibrous dedifferentiation or resorption following ectopic implantation in nude mice[50], consistent with a previous study with synovial MSCs[49]. In contrast, bone marrow MSCs showed increased type X collagen expression and mineralisation, both in response to *in vitro* hypertrophic culture conditions and following *in vivo* ectopic implantation[50], in line with other previous studies with bone marrow MSCs[44,45]. Adipose-derived MSCs[35], although considered an attractive source of MSCs due to the large numbers of cells that can be harvested with relatively little donor morbidity, do not seem to be conducive for cartilage repair[63,66,67]. This may be related to their lack of expression of TGF-β type I receptor and reduced expression of bone morphogenetic protein (BMP)-2, BMP-4, and BMP-6, when compared with bone marrow MSCs[68]. Infrapatellar fat pad-derived MSCs, however, are more chondrogenic and appear similar in potency and functionality to synovial MSCs[50,63,67,69].

Such findings show that, despite similar phenotypic characteristics, MSCs from different tissues show significant functional heterogeneity, both *in vitro* and following *in vivo* transplantation. This may reflect distinct physiological roles in their native tissues. Bone marrow MSCs form an essential part of the bone marrow stroma, supporting and regulating haematopoiesis through interactions with haematopoietic stem cells[70], as well as the sinusoidal network[71]. In addition, they seem to function to continuously replenish the local pool of short-lived osteoblasts[72]. In contrast, MSCs in synovium may primarily function as a reservoir of stem cells for the endogenous regeneration/repair of joint tissues, including articular cartilage and menisci, although this remains to be established. It is possible that imprinted embryonic memory from distinct ontogeny paths is responsible for the variation in biological properties of MSCs from different tissues following their isolation, culture-expansion, and *in vivo* implantation, and possibly also to some extent for their distinct endogenous functions *in vivo*. Alternatively, epigenetic changes may be induced in MSCs according to local environmental cues within their native tissues, which could also be responsible for the observed heterogeneity of MSCs from different tissues.

Heterogeneity of MSCs within tissues

In addition to the heterogeneity between MSCs from different tissues, considerable heterogeneity exists between individual cells isolated from the same tissue. Due to the lack of specific markers to identify human MSCs, these cells are typically isolated based on their plastic-adherence, and defined retrospectively by phenotypic marker expression and ability to differentiate into osteoblasts, chondrocytes and adipocytes[73]. Therefore, MSC cultures are heterogeneous populations of cells that at the single-cell level show significant variability in potency[33,74–76]. On the one hand, this heterogeneity may result in variability in clinical outcomes and highlights the need for robust quality-control of cell preparations, while on the other hand this raises the possibility of identifying and purifying subpopulations of cells with improved potency.

The search for specific markers to purify MSCs prospectively has mostly been explored in bone marrow. A key candidate marker for MSC enrichment that has emerged is CD271, or low-affinity nerve growth factor receptor (LNGFR)[77,78]. The CD271^{bright} population of non-haematopoietic bone marrow cells appears to contain all colony forming cells[78–80], although additional markers are likely required in order to increase MSC purity[81]. One of these that has received attention is CD146 or melanoma cell adhesion molecule (MCAM)[71]. Recent studies investigating expression of both CD271 and CD146 in human bone marrow found CD146 to be expressed by a subpopulation of CD271^{bright} cells[79,80]. Both the CD271^{bright}CD146^{+} and CD271^{bright}CD146^{-} fractions contained colony forming cells that expressed classical MSC markers, including CD73, CD90 and CD105, and were capable of trilineage differentiation[79,80]. Tormin *et al.*[79] further demonstrated that CD146 expression *in vivo* is restricted to perivascular CD271-expressing cells while endosteal CD271-expressing cells lacked CD146 expression, although whether the cells identified *in vivo* are clonogenic, multipotent cells remains to be proven. Interestingly, CD146 expression in culture was upregulated in normoxia and downregulated under hypoxic conditions, suggesting that CD146 expression by MSCs *in vivo* may be variable depending on vicinity to the vasculature and local oxygen levels[79], although other mechanisms such as calcium-induced CD146 shedding on cells close to the bone surface may contribute[82]. Whether functional differences exist between CD271^{bright}CD146^{+} and CD271^{bright}CD146^{-} cells that would favour either one of these populations for different therapeutic applications remains to be clarified.

Delivery method

In addition to the choice of cell populations, the delivery method is also likely to impact on the success of MSC therapy, and many different delivery systems have been explored. Most studies have delivered the cells using a three-dimensional scaffold that is implanted in the defect site, usually by means of an open surgical procedure. Selection of the scaffold material is an important aspect as it can provide topographical cues as well as chemotactic and growth factors. Large joint surface defects not only affect the articular cartilage but also the subchondral bone. The thinning of the articular cartilage goes hand in hand with a thickening and stiffening of the subchondral plate, making the cartilage more susceptible to damage from shear forces. Treatment of large joint surface defects should therefore aim to restore the entire osteochondral unit, not simply the overlying cartilage. To this end, several groups have developed multiphasic osteochondral scaffolds, to mimic osteochondral structures for guiding the repair of both the cartilage and underlying bone, which have shown promising results in preclinical studies[83]. However, regional specification of transplanted MSCs to directly contribute to both articular cartilage and subchondral bone repair appears to take place in the absence of multiphasic scaffolds, at least preclinically[84], suggesting that local environmental cues may be effective in guiding the repair of, and integration with, the subchondral bone. A bigger challenge appears to be stable lateral integration of newly-formed cartilage to existing adjacent cartilage[85]. Several approaches have been explored in *in vitro* or ectopic transplantation models to promote integration of neocartilage with the surrounding cartilage, such as pretreatment with matrix-degrading enzymes[86,87], treatment with an inhibitor of apoptosis to prevent chondrocyte death typically occurring at wound edges[88], or delivery of exogenous chondrocytes to the cartilage interface suspended in a fibrin glue[89] or seeded onto a collagen membrane[90]. How successful such approaches are orthotopically, or indeed clinically, remains to be determined.

Cartilage repair through stimulation of endogenous stem cells and reparative signalling pathways

It is generally thought that articular cartilage has little capacity for intrinsic regeneration and repair, possibly due to a lack of blood supply, low cell mobility due to surrounding matrix, and/or a limited number of progenitor cells. Therefore, tissue engineering

Table I
Comparison of chondrocytes and mesenchymal stem cells for articular cartilage repair

	Chondrocytes	Mesenchymal stem cells
Advantages	• Provide a "like for like" replacement for degenerated cartilage • Proven clinical safety and efficacy	• Easy to isolate from various adult tissues • Easy to culture-expand • Anti-inflammatory as well as regeneration properties • Amenable to one-stop 'off-the-shelf' procedure
Disadvantages	• Need for invasive surgery to harvest cells • Technically challenging to obtain sufficient numbers • Tendency to dedifferentiate during culture-expansion	• Tendency to give rise to hypertrophic cartilage susceptible to calcification and endochondral ossification • Unreliability of cellular products due to heterogeneity and lack of standardised bioprocessing

strategies have been explored and adopted as a means to provide exogenous cartilage tissue. In recent years, it has become increasingly clear, however, that the actions of exogenously administered MSCs go beyond their differentiation potential and the replacement of cells lost due to injury or disease. In the study by Murphy *et al.*, employing intra-articular injection of labelled MSCs in a goat model of trauma-induced OA, it was observed that the injected, labelled bone marrow MSCs did not engraft on the articular cartilage, and only accounted for a minority of the chondrocytes in the regenerated meniscus[37]. In a more recent study, one day after intra-articular injection of GFP-labelled adipose-derived MSCs in a mouse model of OA, GFP-positive cells were found attached to cruciate ligaments, as well as within the synovium, while at 5 days after injection, virtually no GFP-positive cells were observed. Yet, thickening of the synovial lining and formation of enthesophytes were significantly inhibited 42 days after OA induction when MSCs were injected early (at day 7)[91]. Similarly, intraperitoneal injection of MSCs prevented tissue destruction in the mouse model of collagen-induced arthritis, even though injected MSCs were not found to localise to the joints[92].

Although the effects of MSCs for the prevention of OA and inflammatory arthritis through the secretion of trophic factors may be distinct from their ability to directly repair a tissue defect *via* cell replacement, such findings have suggested that MSCs establish a repair-conducive microenvironment, stimulating the recruitment of, and tissue repair by, endogenous stem/progenitor cells. Taking this one step further, this raises the possibility that we may be able to design bioactive scaffolds or therapeutics that elicit an effective tissue repair response in the host through activation and mobilisation of endogenous stem and progenitor cells without the need to administer exogenous cells. Such approaches would be easier to implement clinically and likely to encounter fewer regulatory hurdles.

Several studies have explored the use of scaffolds impregnated with various chemotactic or differentiation factors. Lee *et al.*[93] demonstrated that an acellular scaffold infused with TGF-β3-absorbed collagen hydrogel was able to induce repair of the entire articular surface, as well as the subchondral bone, of proximal humeral condyles in rabbits, with biomechanical properties close to that of native cartilage. However, whether the repair tissue is of equivalent quality to native cartilage, and could be considered regenerated tissue as the authors claim, is questionable judging by the irregular and somewhat fibrotic surface appearance of the repair tissue[93]. Unfortunately, no histomorphological comparison with native cartilage was presented. Nevertheless, the findings from this proof-of-concept study raise the possibility that we may be able to repair an entire joint surface, not just a focal defect in the articular cartilage, through the stimulation of endogenous stem/progenitor cells. More recently, Zhang *et al.*[94] used stromal cell-derived factor-1 (SDF-1) to promote migration of endogenous stem/progenitor cells to the defect site. Repair was achieved in partial-thickness defects of the articular cartilage in rabbit knee joints when a collagen I scaffold with SDF-1 was implanted, whereas defects failed to heal if left untreated or implanted with collagen I alone. Endogenous cells expressing MSC markers were detected along the cartilage surface and within the defect[94]. This interesting study shows the potential to stimulate cartilage repair by using chemotactic factors that recruit endogenous stem/progenitor cells to the defect site. However, the repair tissue that had filled the defect at 6 weeks post-treatment was positive for collagens type I and X, indicative of a fibrous/hypertrophic tissue[94]. In this regard, it should be noted that SDF-1 is not only a chemotactic factor, but also promotes chondrocyte hypertrophy through its interaction with C-X-C chemokine receptor type 4 (CXCR4) on chondrocytes and upregulation of RUNX2 expression, which is part of the cascade of endochondral ossification in the growth plate[95,96]. Hence, SDF-1 may not represent the ideal chemotactic factor. In addition, the recruitment of stem/progenitor cells to the defect site alone may not be sufficient to give rise to stable cartilage repair, and may need to be combined with factors regulating MSC differentiation and induction of a stable chondrocyte phenotype.

While the factors regulating chondrocyte phenotype in the growth plate are now well-understood, the factors responsible for the formation and maintenance of stable articular cartilage are still poorly defined. Recently, Johnson *et al.* identified the small molecule kartogenin as a stimulator of chondrogenic differentiation of bone marrow MSCs, acting *via* a novel biological pathway involving binding to filamin A and disrupting its interaction with the transcription factor core-binding factor b subunit (CBFb)[97]. Intra-articular injection of kartogenin was found to protect against OA development in mice, and it was suggested that kartogenin may modulate endogenous stem cells to confer a regenerative/repair effect and/or a protective effect. Kartogenin may also protect existing chondrocytes against the pathological effects of proinflammatory cytokines[97]. This study thus shows the potential to identify new biological pathways and small molecules that promote joint homoeostasis and protect against OA.

Conclusions and future perspective

Arthroplasty remains the ultimate intervention for end-stage OA patients with chronic pain and functional disability. However, for pre-OA joint surface lesions and early OA, cell-based therapies are increasingly becoming available. The type of cell-based therapeutic intervention will likely depend on the clinical indication and on factors such as size and depth of the lesion, health status of the surrounding articular cartilage and of the other joint tissues, and will range from ACI (with chondrocytes, MSCs or other stem cell types) to the implantation of cell-free scaffolds loaded with regenerative biomolecules.

MSCs represent promising types of adult stem cells for joint surface repair, offering both advantages and disadvantages compared with autologous chondrocytes (Table I). Besides their capacity to differentiate into chondrocytes and osteoblasts, they play a role as cellular modulators to endorse tissue repair *via* secretion of bioactive molecules. Intriguingly, the MSC capacity to activate endogenous reparative mechanisms appears to be dependent on the degree of

stemness[98], highlighting the importance of consistent bioprocessing of the MSC products to deliver to patients. There is therefore a requirement for cell potency assessment in order to standardise manufacturing and ensure clinical effectiveness.

The availability of large batches of "off-the-shelf" quality-controlled allogeneic MSC populations will enhance consistency of treatments while reducing costs; it will also eliminate the need for two operations and enable large-scale production. Alternatively, several devices are becoming available to purify from bone marrow cell populations containing MSCs, to be then implanted straight-away into the patient's cartilage lesion in a one-stop procedure. Minimally manipulated MSC preparations are expected to simplify autologous procedures as well as the regulatory paths.

Current therapeutic approaches to joint surface defects pursue long-lasting, pain-free joint function by promoting the formation of a repair tissue that integrates with the surrounding tissues and displays a cell phenotype, extracellular matrix composition and tissue durability that are similar to articular cartilage. Regenerative medicine aims at biological regeneration of the damaged joint tissues to fully restore joint homoeostasis, regeneration referring to the replacement of damaged tissue with an identical tissue such that both anatomy and function are fully restored. Regeneration could follow the initial phase of repair through long-lasting remodelling, as occurs in highly regenerative tissues such as the skin. There is some evidence to suggest that cartilage remodelling and, possibly, regeneration can occur in at least some patients undergoing ACI[24]. Whether regeneration may be achieved using MSC implantation is at present unclear.

A fascinating prospect is the pharmacological targeting, by using drugs, of the native stem cells and related reparative signalling pathways in the joint in order to repair or even regenerate the damaged joint tissues. In this regard, there is evidence that, under specific conditions, the joint surface has healing potential as reported in animal models[93,99] and in humans[100], but the mechanisms remain to be elucidated. Hence, the identification and characterisation of the stem cell niches in the joint and the investigation of how signals at the niche sites are orchestrated towards joint homoeostasis, remodelling and repair are important areas of research. The lack of specific markers to identify MSCs in their native tissues *in vivo* has for a long time hampered studies aimed at understanding the roles of MSCs during tissue homoeostasis, remodelling and repair. However, with the discovery of novel markers[70–72,79] and alternative approaches to identify functional MSCs *in vivo*[101], we are beginning to elucidate the *in vivo* location of MSCs and characterise the niches that regulate MSC fate and function. The cell-based biological therapies of the joint tissues appear to be just at the beginning of their journey but have the potential to benefit millions of patients worldwide.

Author contributions

AJR and CDB designed the study. All authors contributed to the collection and assembly of data, analysis and interpretation of data, writing of the manuscript and final approval of the article.

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Competing interest statement

The authors have no conflict-of-interest to disclose.

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